FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS' ENTERED AT 15:09:59 ON 30 MAR 2008

L1 130231 S (ESTROGEN RECEPTOR) OR (ER ALPHA) OR (ER BETA)
L2 1212 S L1 (S) FUSION
L3 10 S L1 (S) (SINGLE CHAIN)
L4 9782 S L2 AND (ESTROGEN RESPONSE LEMENT) OR (ERE)
L5 30 S L2 AND ((ESTROGEN RESPONSE LEMENT) OR (ERE))
L6 6 DUP REM L3 (4 DUPLICATES REMOVED)
L7 21 DUP REM L5 (9 DUPLICATES REMOVED)

AU Muyan, M. (correspondence); Yi, P.; Sathya, G.; Willmert, L.J.; Driscoll, M.D.; Hilf, R.; Bambara, R.A.
CS Department of Biochemistry, Univ. of Rochester Medical Center, Rochester, NY 14642, United States. mesut_muyan@urmc.rochester.edu
SO Molecular and Cellular Endocrinology, (20 Sep 2001) Vol. 182, No. 2, pp. 249-263.
Refs: 56
ISSN: 0303-7207 CODEN: MCEND6
TI Fusion estrogen receptor proteins: Toward the development of receptor-based agonists and antagonists.
AB Estrogen-induced signaling mediated by estrogen receptors (ERs) is also affected by aberrant ERs that act as constitutively active or dominant negative modulators. Variant ERs can contribute to carcinogenesis and to the loss of estrogen responsiveness, rendering antiestrogen therapy ineffective. Determining target gene response during co-synthesis of different ER species is difficult, because dimers formed in the presence of more than one ER species are a heterogenous population of homo- or heterodimers. We engineered a homofusion ER.alpha. as a prototype single-chain receptor by genetically conjugating two ER monomers into a covalently fused single-chain protein to obtain a homogeneous population. This permits analysis of symmetrical or asymmetrical mutations that simulate variant homo- and heterodimers. Although a monomer, the homofusion receptor exhibited similar biochemical and functional properties to the dimeric ER.alpha.. We used activation function-2 (AF2) defective mutants as a model in either one or both receptor domains for a dominant-negative phenotype by suppressing the reporter activity induced by the WT receptor. When co-expressed with ER.alpha., the fusion variant deficient in both AF2 functions suppressed the reporter activity effectively induced by ER.alpha.. These results show the utility of fusion receptors as models for generation of receptor-based agonists and antagonists. .COPYRGT. 2001 Elsevier Science Ireland Ltd. All rights reserved.

AU Li Xiaodong; Huang Jing; Yi Ping; Bambara Robert A; Hilf Russell; Muyan Mesut
SO Molecular and cellular biology, (2004 Sep) Vol. 24, No. 17, pp. 7681-94. Journal code: 8109087. ISSN: 0270-7306.
TI Single-chain estrogen receptors (ERs) reveal that the ERalpha/beta heterodimer emulates functions of the ERalpha dimer in genomic estrogen signaling pathways.
AB The effects of estrogens, particularly 17beta-estradiol (E2), are mediated by estrogen receptor alpha (ERalpha) and ERbeta. Upon binding to E2, ERs homo- and heterodimerize when coexpressed. The ER dimer then regulates the transcription of target genes through estrogen responsive element (ERE)-dependent and -independent pathways that constitute genomic estrogen signaling. Although ERalpha and ERbeta have similar ERE and E2 binding properties, they display different transregulatory capacities in both ERE-dependent and -independent signaling pathways. It is therefore likely that the heterodimerization provides novel functions to ERs by combining distinct properties of the contributing partners. The elucidation of the role of the ER heterodimer is critical for the understanding of physiology and pathophysiology of E2 signaling. However, differentially determining target gene responses during cosynthesis of ER subtypes is difficult, since dimers formed are a heterogeneous population of homo- and

heterodimers. To circumvent the pivotal dimerization step in ER action and hence produce a homogeneous ER heterodimer population, we utilized a genetic fusion strategy. We joined the cDNAs of ERalpha and/or ERbeta to produce single-chain ERs to simulate the ER homo- and heterodimers. The fusion ERs interacted with ERE and E2 in a manner similar to that observed with the ER dimers. The homofusion receptors mimicked the functions of the parent ER dimers in the ERE-dependent and -independent pathways in transfected mammalian cells, whereas heterofusion receptors emulated the transregulatory properties of the ERalpha dimer. These results suggest that ERalpha is the functionally dominant partner in the ERalpha/beta heterodimer.

AU Demirpence Ediz; Semlali Abdelhabib; Oliva Joan; Balaguer Patrick; Badia Eric; Duchesne Marie-Josephe; Nicolas Jean-Claude; Pons Michel
SO Cancer research, (2002 Nov 15) Vol. 62, No. 22, pp. 6519-28.
Journal code: 2984705R. ISSN: 0008-5472.
TI An estrogen-responsive element-targeted histone deacetylase enzyme has an antiestrogen activity that differs from that of hydroxytamoxifen.
AB We showed previously that prolonged treatment of a MCF-7-derived cell line with hydroxytamoxifen (OHT) induces the irreversible silencing of some estrogen-responsive genes, whereas OHT-resistant cell growth appears simultaneously (E. Badia et al., Cancer Res., 60: 4130-4138, 2000). Based on the hypothesis that particular gene silencings could be involved in triggering the resistance phenomenon, we focused our study on the mechanism of OHT-induced silencing. More precisely, we wished to determine to what extent the recruited histone deacetylase (HDAC) activity, which is known to be involved in the repressive effect induced by antagonist ligands of nuclear receptors, could participate in various aspects of OHT effects, particularly in gene silencing. A fusion protein (HDAC-EG) of human HDAC1 fused with the estrogen receptor DNA-binding domain and the glucocorticoid receptor ligand-binding domain allowed targeting of chimeric HDAC1 activity on estrogen-responsive elements (EREs) in the presence of glucocorticoid ligands. When HDAC-EG was transiently expressed in HeLa cells together with estrogen receptor, an antiestrogen-like effect was obtained on an ERE-controlled luciferase reporter gene in the presence of agonist or antagonist glucocorticoids. In MCF-7-derived cells stably expressing HDAC-EG and an estrogen-regulated luciferase, liganded HDAC-EG again produced an antiestrogenic effect on expression of natural estrogen-regulated genes such as pS2, progesterone receptor, and cathepsin D and cell growth together with chimeric luciferase gene expression. However, a prolonged HDAC-EG-mediated antiestrogen effect did not lead to irreversible luciferase gene silencing, as OHT does. It nevertheless accelerated the OHT-driven phenomenon. The antiestrogen effect of OHT thus differs from that of an ERE-targeted HDAC1 activity that might participate in irreversible silencing but is not sufficient to trigger it.

AU Schultz, Jennifer R.; Loven, Margaret A.; Melvin, Vida M. Senkus; Edwards, Dean P.; Nardulli, Ann M.
SO Journal of Biological Chemistry (2002), 277(10), 8702-8707
CODEN: JBCHA3; ISSN: 0021-9258
TI Differential modulation of DNA conformation by estrogen receptors .alpha. and .beta.
AB The human estrogen receptor (ER) induces transcription of estrogen-responsive genes upon binding to estrogen and the estrogen response element (ERE). To det. whether receptor-induced changes in DNA structure are related to transactivation, we compared the abilities of ER.alpha. and ER.beta. to activate transcription and induce distortion and bending in DNA. ER.alpha. induced higher levels of transcription than ER.beta. in the presence of 17.beta.-estradiol. In circular permutation expts. ER.alpha.

induced greater distortion in DNA fragments contg. the consensus ERE sequence than ER.beta.. Phasing anal. indicated that ER.alpha. induced a bend directed toward the major groove of the DNA helix but that ER.beta. failed to induce a directed DNA bend. Likewise, the ER.alpha. DNA binding domain (DBD) and hinge region induced a bend directed toward the major groove of the DNA helix, but the ER.beta. DBD and hinge region failed to bend ERE-contg. DNA fragments. Using receptor chimeras we demonstrated that the ER.alpha. DBD C-terminal extension is required for directed DNA bending. Transient transfection assays revealed that appropriately oriented DNA bending enhances receptor-mediated transactivation. The different abilities of ER.alpha. and ER.beta. to induce change in DNA structure could foster or inhibit the interaction of regulatory proteins with the receptor and other transcription factors and help to explain how estrogen-responsive genes are differentially regulated by these two receptors.

IN Mao, Chengjian; Shapiro, David J.
SO U.S. Pat. Appl. Publ., 94 pp.
CODEN: USXXCO
TI Estrogen receptor fusion proteins in a tamoxifen-inducible expression system for eukaryotic cells
AB An expression system inducible by tamoxifen or its analogs and repressible by estrogen analogs such as ICI 182780 uses fusion proteins of estrogen receptors and transcription factors such as VP16 to control transcription through an estrogen-responsive element. Such systems have a wide variety of applications, including gene therapy and in vivo and in vitro expression, as well as their use in transgenic animals. A series of derivs. of estrogen receptor .alpha. with changes in the sequence of the zinc finger at amino acids 203-219 were prepd. by std. methods and used in fusion proteins with the C-terminal transactivation domain of VP16. The fusion proteins bound to variants of the estrogen-responsive element (ERE). Binding of the fusion protein to an ERE was stimulated by potent estrogens such as moxestrol and 4-hydroxytamoxifen. A series of known mutations in the estrogen receptor were tested for their effects on transactivation and used to design a tightly regulated induction system. Synthetic promoters contg. multiple copies of an estrogen responsive element are also described. Variants of the system showing different degrees of basal expression and inducibility are described.

AU Buluwela L; Pike J; Mazhar D; Kamalati T; Hart S M; Al-Jehani R; Yahaya H; Patel N; Sarwar N; Sarwarl N; Heathcote D A; Schwickerath O; Phoenix F; Hill R; Aboagye E; Shousha S; Waxman J; Lemoine N R; Zelent A; Coombes R C; Ali S
SO Gene therapy, (2005 Mar) Vol. 12, No. 5, pp. 452-60.
Journal code: 9421525. ISSN: 0969-7128.
TI Inhibiting estrogen responses in breast cancer cells using a fusion protein encoding estrogen receptor -alpha and the transcriptional repressor PLZF.
AB Estrogen receptor alpha (ERalpha) is a ligand-inducible transcription factor that acts to regulate gene expression by binding to palindromic DNA sequence, known as the estrogen response element, in promoters of estrogen-regulated genes. In breast cancer ERalpha plays a central role, where estrogen-regulated gene expression leads to tumor initiation, growth and survival. As an approach to silencing estrogen-regulated genes, we have studied the activities of a fusion protein between ERalpha and the promyelocytic leukemia zinc-finger (PLZF) protein, a transcriptional repressor that acts through chromatin remodeling. To do this, we have developed lines from the estrogen-responsive MCF-7 breast cancer cell line in which the expression of the fusion protein PLZF-ERalpha is conditionally regulated by tetracycline and shows that these feature long-term silencing of the

expression of several well-characterized estrogen-regulated genes, namely pS2, cathepsin-D and the progesterone receptor. However, the estrogen-regulated growth of these cells is not inhibited unless PLZF-ERalpha expression is induced, an observation that we have confirmed both in vitro and in vivo. Taken together, these results show that PLZF-ERalpha is a potent repressor of estrogen-regulated gene expression and could be useful in distinguishing estrogen-regulated genes required for the growth of breast cancer cells.

IN Muyan, Mesut; Huang, Jing
SO PCT Int. Appl., 205 pp.
CODEN: PIXXD2
TI Chimeric hormone response element binding transregulators and use as antitumor agents
AB The invention discloses compns. and methods for ERE (estrogen response element)-binding transregulators that specifically and potently regulate ERE -contg. genes. To accomplish this, the authors took advantage of the modular nature of estrogen receptor and initially designed a monomeric ERE binding module by co-joining two DNA binding domains with the hinge domain. Integration of strong activation or repressor domains from other transcription factors into this module generated constitutively active ERE-binding activators (EBAs) and ERE-binding repressors (EBRs) resp. These transregulators are the basis for the targeted regulation of ERE contg. genes, the identification of estrogen responsive gene networks, and the development of alternative/complementary therapeutic approaches for estrogen target tissue cancers. An example of the invention describes EBAs, such as estrogen receptor .alpha. CDC domain fusions with VP16 activation domain or NF-.kappa.B p65 subunit activation domain, that induced expression of only ERE-contg. genes independent of ligand binding, dimerization, ER subtypes, promoter- and cell-context. The EBAs differently altered cell cycle progression in cells derived from breast cancer. The EBAs increased the no. of cells in G1 phase of ER-neg. MDA-MB-231 cells and decreased the no. of cells in G1 phase in ER-pos. MCF-7 cells.

AU Chien P Y; Ito M; Park Y; Tagami T; Gehm B D; Jameson J L
SO Molecular endocrinology (Baltimore, Md.), (1999 Dec) Vol. 13, No. 12, pp. 2122-36.
Journal code: 8801431. ISSN: 0888-8809.
TI A fusion protein of the estrogen receptor (ER) and nuclear receptor corepressor (NCoR) strongly inhibits estrogen-dependent responses in breast cancer cells.
AB Nuclear receptor corepressor (NCoR) mediates repression (silencing) of basal gene transcription by nuclear receptors for thyroid hormone and retinoic acid. The goal of this study was to create novel estrogen receptor (ER) mutants by fusing transferable repressor domains from the N-terminal region of NCoR to a functional ER fragment. Three chimeric NCoR-ER proteins were created and shown to lack transcriptional activity. These fusion proteins silenced basal transcription of the ERE2-tk-Luc reporter gene and inhibited the activity of co-transfected wild-type ER (wtER), indicating that they possess dominant negative activity. One of the fusion proteins (CDE-RD1), containing the ER DNA-binding and ligand-binding domains linked to the NCoR repressor domain (RD1), was selected for detailed examination. Its hormone affinity, intracellular localization, and level of expression in transfected cells were similar to wtER, and it bound to the estrogen response element (ERE) DNA in gel shift assays.
Glutathione-S-transferase pull-down assays showed that CDE-RD1 retains the ability to bind to steroid receptor coactivator-1. Introduction of a DNA-binding domain mutation into the CDE-RD1 fusion protein eliminated silencing and dominant negative activity. Thus, the RD1 repressor domain

prevents transcriptional activation despite the apparent ability of CDE-RD1 to bind DNA, ligand, and coactivators. Transcriptional silencing was incompletely reversed by trichostatin A, suggesting a histone deacetylase-independent mechanism for repression. CDE-RD1 inhibited ER-mediated transcription in T47D and MDA-MB-231 breast cancer cells and repressed the growth of T47D cells when delivered to the cells by a retroviral vector. These ER-NCoR fusion proteins provide a novel means for inhibiting ER-mediated cellular responses, and analogous strategies could be used to create dominant negative mutants of other transcription factors.